Direct Screening Methods for Rapid Identification of KPC-Producing Klebsiella Pneumoniae and Escherichia coli

Thesis

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طريقة الفحص المباشر للتعرف على كلبسيلا الإلتهاب الرئوي والإشريشية القولونية المنتجين لكاربابينواميز كلبسيلا الإلتهاب الرئوي في العينات الإكلينيكية

رسالـــة

توطئة للحصول على درجة الماجستير في الباثولوجيا الإكلينيكية والكيميائية

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مدرس الباثولوجيا الإكلينيكية والكيميائية

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List of Abbreviations

ABC	Adenosine triphosphate (ATP)-binding cassette
AcrAB	Acriflavine resistance protein A and B
AK	Amikacin
ALG	Alginate
AMC	Amoxicillin- clavulanic acid
AmpC	Ambler Class C
APB	aminophenyl-boronic acid
BA-CD	Boronic acid combined disc test
bla	Beta -lactamase
BMD	Broth microdilution
CAZ	Ceftazidime
CDC	Center for Disease Control and Prevention
CFP	Cefoperazone
CIP	Ciprofloxacin
CLSI	Clinical Laboratory Standards Institute
CM	Cytoplasmic membrane
CP	Carbapenemase-producing
CPD	Cefpodoxime
CRO	Ceftriaxone
CTX	Cefotaxime
CTX-Ms	Cefotaximase
ddNTP	dideoxynucleotide triphosphate
DDST	Double disc synergy test
E. coli	*Escherichia coli*
E-test	Epsilometer test
EDTA	Ethylene-diamine-tetra-acetic acid
ESBLs	Extended-spectrum β lactamases
EU	European Union
F	Nitrofurantoin

FOX	Cefoxitin
GES	Guiana extended spectrum beta- lactamase
GIM	German imipenemase
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
IBC	Integron borne cephalosporinase
ICU	Intensive care unit
IEF	Isoelectric focusing
IM	inner membrane
IMI	Imipenem hydrolyzing β- lactamase
IMP	Inner membrane proteins
IND	indologenes
IPM	Imipenem
K.	*Klebsiella*
KPC	*Klebsiella pneumoniae* carbapenemase
LPS	Lipopolysaccharide
MATE	Multidrug and toxic compound extrusion
MBLs	Metallo-ß-lactamases
MB-PCR	molecular beacons-polymerase chain reaction
MDR	Multi drug resistant
MEM	Meropenem
MFP	membrane fusion protein
MFS	Major facilitator superfamily
MHT	Modified Hodge Test
MIC	Minimum inhibitory concentration
MRSA	Methicillin resistant *Staphylococcus aureus*
NDM	New Delhi metallo-ß-lactamase
NMC	Non metalloenzyme carbapenemase
OM	Outer membrane
OMP	Outer membrane proteins
OmpC	Outer membrane protein C

OmpF	Outer membrane protein F
Omp K35	Osmoporins of *klebsiella pneumoniae*
OmpK36	Osmoporins of *klebsiella pneumoniae*
OXA	Oxacillin
OXA-MHT	Oxacillin –Modified Hodge Test
P.	*Pseudomonas*
PBPs	Penicillin Binding Proteins
PC1	penicillinases 1
PCR	Polymerase Chain Reaction
PFGE	Pulsed- Field Gel Electrophoresis
PG	Peptidoglycan
pIs	Isoelecteric points
Qnr	quinolones resistance
RND	Resistance-nodulation-cell-division
S.	*Streptococcus*
S. marcescens	*Serratia marcescens*
SHV	sulfhydryl variable
SIM	Seoul imipenemase
SME	*Serratia marcescens* enzyme
SMR	Small multidrug resistance
SPM	San Paulo metallo-β-lactamase
Spp.	Species
SXT	Trimethoprim-Sulfamethoxazole
TEM	Temoneira (name after the patient providing the first sample)
TZP	Tazobactam
US	United States
UTI	Urinary tract infection
VIM	Verona integron-encoded metallo-β-lactamase
Zn	Zinc

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✍ ***Walaa Ahmed Barakat***


بسم الله الرحمن الرحيم
قَالُوا سُبْحَانَكَ لاَ عِلْمَ لَنَا
إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ
صدق الله العظيم
سورة البقرة الآية (٣٢)

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Introduction

The growing increase in the rates of antibiotic resistance is a major cause for concern in both nonfermenting bacilli and isolates of the Enterobacteriaceae family. β-Lactams have been the mainstay of treatment for serious infections, and the most active of these are the carbapenems, which are advocated for use for the treatment of infections caused by extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, particularly *Escherichia coli (E.coli)* and *Klebsiella pneumoniae (K. pneumoniae)* ***(Kaul and Chhina, 2010).***

Carbapenem resistance among Enterobacteriaceae, in particular *K. pneumoniae* and *E.coli*, is an emerging problem worldwide. Several resistance mechanisms have been reported to circumvent the efficacy of carbapenems, and carbapenemases (carbapenem- hydrolyzing β-lactamases) are the most prominent enzymes that neutralize carbapenems. Class A carbapenemases, which include bla_{KPC}, NMC, SME-1 to -3, IMI-1, and GES, have been characterized in several genera of the family Entero-bacteriaceae ***(Wang et al., 2012).***

Klebsiella pneumoniae carbapenemase (KPC) is a molecular class A serine β-lactamase belonging to functional group 2f ***(Fontana et al., 2010).***The KPC β-lactamase occurs most commonly in *K. pneumoniae,* but it has also been reported

sporadically in other species of Enterobacteriaceae *(Klebsiella oxytoca, Enterobacter spp., E.coli, Salmonella spp., Citrobacter freundii, and Serratia spp.)* and *Pseudomonas aeruginosa (P.aeruginosa)*. The KPC enzyme confers resistance to all β-lactam agents including penicillins, cephalosporins, monobactams, and carbapenems ***(Francis et al., 2012).***

The patient groups most likely to acquire KPC-producing bacteria include the patients at risk for infections caused by multidrug resistant organisms: patients with invasive devices, prolonged hospital stays (especially in an ICU), and heavy antibiotic exposure and those who are immunocompromised ***(Arnold et al., 2012).***

The bla_{KPC} gene is plasmid mediated and is carried in a Tn3-based transposon, Tn4401; the potential ease of mobility of this resistance mechanism is a major concern ***(Kitchel et al., 2009).*** This plasmid also, often contains genes that code for resistance to non β-lactam agents such as aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole ***(Wang et al., 2012).***Therefore, it is important to isolate infected patients and take contact precautions because of the potential for nosocomial transmission ***(Toye et al., 2009).*** Also, controlled antibiotic usage must be complemented by the utilization of rapid and sensitive bla_{KPC} diagnostic assays ***(Hindiyeh et al., 2008).***